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Pharmacological analysis by HOE642 and KB-R9032 of the role of Na^+/H^+ exchange in the endothelin-1-induced Ca^{2+} signalling in rabbit ventricular myocytes

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1 The role of Na^+/H^+ exchange in endothelin-1 (ET-1)-induced increases in Ca^{2+} transients and cell shortening was studied in rabbit ventricular myocytes loaded with indo-1/AM. Selective inhibitors of Na^+/H^+ exchange HOE642 (4-isopropyl-3-methyl-sulphonylbenzoyl guanidine methanesulphonate) and KB-R9032 (N-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2H-benzo-[1,4]oxazine-6-carbonyl) guanidine methanesulphonate) were used as pharmacological tools for the analysis.

2 ET-1 at 0.1 nM induced an increase in Ca^{2+} transients by 45.6%, while it increased cell shortening by 109.6%. For a given increase in cell shortening, the ET-1-induced increase in Ca^{2+} transients was much smaller than that induced by isoprenaline (ISO, 10 nM).

3 Pretreatment with HOE642 and KB-R9032 (1 μM) inhibited the increase in cell shortening induced by 0.1 nM ET-1 by 51 and 65.4%, respectively, without a significant alteration of ET-1-induced increase in Ca^{2+} transients. HOE642 and KB-R9032 did not affect baseline levels of cell shortening and peak Ca^{2+} transients, and the effects of ISO (10 nM).

4 These results indicate that activation of Na^+/H^+ exchange by ET-1 may play an important role in the positive inotropic effect and the ET-1-induced increase in myofilament Ca^{2+} sensitivity in rabbit ventricular myocytes.

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Abbreviations: DAG, 1,2-diacylglycerol; ET-1, endothelin-1; HEPES, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid]; indo-1/AM, acetoxymethylester of indo-1; IP_3, inositol 1,4,5-trisphosphate; ISO, isoprenaline; K-H, Krebs-Henseleit; pH_i, intracellular pH; PI, phosphoinositide; PIE, positive inotropic effect; PKC, protein kinase C

Introduction

Endothelin-1 (ET-1) is a vasoactive polypeptide with 21-amino acids that was originally isolated from the culture medium of porcine aortic endothelial cells (Yanagisawa *et al*., 1988). In mammalian cardiac muscle of various species, ET-1 has also a pronounced positive inotropic effect (PIE) (Ishikawa *et al*., 1988; Hu *et al*., 1988; Takanashi & Endoh, 1992; Moravec *et al*., 1989). The PIE of ET-1 has been shown to be associated with an activation of phospholipase C and the resultant acceleration of hydrolysis of phosphoinositide (PI) that leads to production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (Berridge, 1993; Hansen *et al*., 1995; Meyer *et al*., 1996). In the heart, pieces of experimental evidence imply that activation of protein kinase C (PKC) by DAG that is generated endogenously by ET-1 is coupled to divergent regulatory processes, including facilitation of various types of ion channel and ion transport system, that leads to characteristic modulation of intracellular Ca^{2+} signalling at the final step of cardiac contraction (Krämer *et al*., 1991; Yang *et al*., 1999).

In cardiac muscle, Na^+/H^+ exchange plays a crucial role to maintain the intracellular pH (pH_i) within a physiological range by extruding protons in exchange for Na^+ ions, activation of which leads to intracellular alkalinization and accumulation of Na^+ ions. Since ET-1 as well as phenylephrine and angiotensin II stimulate the PI hydrolysis, it has been postulated that the activation of PKC-dependent Na^+/H^+ exchanger and resultant intracellular alkalinization might be a potential mechanism for the PIE of these receptor agonists (Krämer *et al*., 1991; Matsui *et al*., 1995; Yokoyama *et al*., 1998). Intracellular alkalinization has been shown to enhance the myofilament sensitivity to Ca^{2+} ions in skinned cardiac fibres (Fabiato & Fabiato, 1978) and supposed to play an important role in an increase in myocardial contractility in intact myocardium (Bountra & Vaughan-Jones, 1989). The mechanisms responsible for the contractile regulation induced by ET-1 in intact cardiac muscle, however, remains controversial.

The present study was designed to elucidate the role of Na^+/H^+ exchanger in the ET-1-induced PIE in intact myocardium. For this purpose, we examined the influence of novel selective inhibitors of Na^+/H^+ exchanger, HOE642 (4-isopropyl-3-methyl-sulphonylbenzoyl guanidine methanesulphonate) and KB-R9032 [N-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2H-benzo-[1,4]oxazine-6-carbonyl) guanidine methanesulphonate], on the ET-1-induced modulation of Ca^{2+} signalling in adult rabbit ventricular myocytes loaded by indo-1/AM. For comparison, the influence of these compounds on the response to isoprenaline (ISO) that is not associated with alteration of pH_i (Gambassi *et al*., 1992) was also investigated.

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Methods

Isolation of rabbit ventricular myocytes

Rabbit ventricular cardiomyocytes were isolated by means of procedure that has been described previously (Fujita & Endoh, 1996). Briefly, adult male Japanese White rabbits (1.8–2.2 kg) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.v.). The heart was excised and perfused by Langendorff method with Tyrode solution that contains HEPES (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid]) for about 1 min at 37°C to washout blood in the heart. HEPES-Tyrode solution contained (in mM): NaCl, 136.5; KCl, 5.4; $MgCl_2$, 0.53; $CaCl_2$, 1.8; NaH_2PO_4, 0.33; glucose, 5.0; HEPES, 5.0 (pH 7.4) and was continuously bubbled with 100% O_2. Then, the heart was perfused with nominally Ca^{2+} free HEPES-Tyrode solution for 5 min, followed by perfusion with solution containing 0.6 mg ml^{-1} collagenase (class II; Worthington Biochemical, Freehold, NJ, U.S.A.) and 0.1 mg ml^{-1} protease (type XIV; Sigma Chemical Co., St. Louis, MO, U.S.A.) for 16–20 min. Finally, the heart was washed with HEPES-Tyrode solution containing 0.2 mM $CaCl_2$ and ventricles were cut into small pieces. The cell suspension was rinsed several times with a gradual increase in the Ca^{2+} concentration up to 1.8 mM. The myocytes displaying rod shaped, well defined striations and no spontaneous contractions were used for the experiments.

Loading of myocytes with indo-1/AM

Myocytes were loaded with a fluorescent dye acetoxymethyl-ester of indo-1 (indo-1/AM) by incubating them in 5 μM indo-1 solution for about 3 min at room temperature. The loading solution consisted of 10 μl of 1 mM indo-1/AM, 40 μl DMSO, 90 μl foetal bovine serum, 10 μl of 20% (w w^{-1} in DMSO) pluronic F-127 and 1 ml HEPES-Tyrode solution. The loading solution described above was sonicated for 3 min and 1 ml of cell suspension was added to it. After loading, they were centrifuged at about $5 \times g$ for 1 min. The supernatant was discarded and the pellet was resuspended in HEPES-Tyrode solution and stored at room temperature (25–27°C) until they are used for the experiments.

Measurements of cell shortening and Ca^{2+} transients

Myocytes were laid in the chamber that contained Krebs-Henseleit (K-H) bicarbonate buffer and was placed on the stage of an inverted microscope (Diaphot TMD 300; Nikon, Tokyo, Japan) and they were allowed to settle down to attach loosely to the bottom of the chamber for 10 min. Then the perfusion was started with K-H bicarbonate buffer at a rate of 1 ml min^{-1} at room temperature (25–27°C) and the cells were stimulated electrically by square-wave pulses with voltage about 30–40% above the threshold at a frequency of 0.5 Hz. The K-H bicarbonate buffer contained (in mM): NaCl, 116.4; KCl, 5.4; $MgSO_4$, 0.8; $CaCl_2$, 1.8; NaH_2PO_4, 1.0; $NaHCO_3$, 23.8 and glucose, 5.0 (pH 7.4) and had been equilibrated with 95% O_2 and 5% CO_2.

Fluorescence of indo-1 was excited with the light from a xenon lamp (150 W) at a wavelength of 355 nm, reflected by a 380 nm long-pass dichroic mirror, and detected by a fluorescence spectrophotometer (CAM-230; Japan Spectroscopic Co., Tokyo, Japan). Excitation light was applied to myocytes intermittently through a neutral density filter to minimize the photobleaching of indo-1. The emitted fluorescence was collected by an objective lens (CF Fluor DL40, Nikon, Japan) and then separated by a 580 nm long-pass dichroic mirror. The fluorescence light was subsequently split with a 425 nm dichroic mirror to permit simultaneous measurements of light at both 405 and 500 nm wavelengths through band-pass filters.

The emission field was restricted to a single cell with the aid of an adjustable window. The fluorescence ratio (405/500 nm) was used as an indicator of $[Ca^{2+}]_i$ (Grynkiewicz *et al*., 1985). Cells were simultaneously illuminated with red light (wavelength above 620 nm) through the normal bright-field illumination optics of the microscope and the bright-field images of a myocyte were collected by objective lens and first separated by a 580-nm long-pass dichroic mirror (Omega Optical, Brattleboro, VT, U.S.A.). A bright-field cell image was projected onto a photodiode array of the edge detector (C6294-01, Hamamatsu Photonics K.K., Hamamatsu, Japan) with 5 ms temporal resolution and the cell length was monitored simultaneously with indo-1 fluorescence.

Experimental protocols

After an equilibration period of 40 min, myocytes were perfused with the solution containing the agent examined. When the response of the myocytes to the agent applied reached a stable level, the indo-1 fluorescence was measured and then the perfusion was switched to the solution that contained an additional agent. The cell length was continuously measured throughout the experiments, while the fluorescence of indo-1 was monitored intermittently to reduce the quenching. The cell length and Ca^{2+} transients were simultaneously recorded at the baseline and in the presence of the agent examined when the response reached a steady level. ET-1 was applied only once to each preparation, since the effect of ET-1 was not reversible by washout. Selective inhibitors of Na^+/H^+ exchanger were allowed to act for 20 min prior to administration of ET-1 or ISO and were present throughout the experiments.

Data recording and analysis

Cell length and fluorescence of indo-1 were stored and displayed by means of a computer (Power Macintosh 8100/100AV, Apple Computer Inc., Cupertino, CA, U.S.A.) equipped with an A/D converter (MP-100A, BIOPAC Systems Inc., Santa Barbara, CA, U.S.A) at 200 Hz and analysed after low-pass filtering (cutoff frequency of 20 Hz). The data used for statistical analysis were obtained by signal-averaging of five successive tracings of cell shortening and Ca^{2+} transients. In the analysis of data, the systolic cell shortening and indo-1 fluorescence ratio prior to first application of the agent examined in individual experiments were regarded as basal values in each myocyte and are assigned to 100%, and all the data are expressed as a percentage of the basal values.

Drugs

The drugs and reagents used were endothelin-1 (Peptide Institute, Osaka, Japan), (−)-isoprenaline hydrochloride and protease type XIV (Sigma Chem. Co., St. Louis, MO, U.S.A.); indo-1/AM (Dojindo Chemical, Kumamoto, Japan); collagenase (Worthington Biochemical, Freehold, NJ, U.S.A.); HOE642 (4-isopropyl-3-methysulphonylbenzoyl guanidine methanesulphonate; Hoechst Marion Roussel, Tokyo, Japan); KB-R9032 (N-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2H-benzo-[1,4]oxazine-6-carbonyl) guanidine methanesulphonate; Kanebo, Osaka, Japan). Other reagents used were of the highest grade in purity that were commercially available.

Statistical analysis

Data are expressed as means ± s.e.mean. Significance of differences between two mean values was estimated by Student's *t*-test. Differences were considered to be significant when a *P* value is less than 0.05.

Results

Effects of ET-1 and isoprenaline

ET-1 at a concentration of 0.1 nM induced increases in the amplitude of Ca^{2+} transient (indo-1 fluorescence ratio) and the peak cell shortening in a single rabbit ventricular myocyte (Figure 1a). A marked increase in cell shortening was associated with a relatively small enhancement of the amplitude of Ca^{2+} transients. ET-1 at 0.1 nM slightly prolonged the duration of cell shortening with no detectable alteration of duration of Ca^{2+} transient (Figure 1b). Washout of ET-1 for 15 min did not reverse the effect of ET-1 (Figure 1a). On average, diastolic indo-1 ratio and diastolic cell length were not significantly affected by ET-1 at 0.1 nM (Table 1), while the systolic indo-1 ratio and systolic cell shortening in these myocytes were increased significantly: when the basal values were assigned to 100%, systolic indo-1 ratio was 145.6 + 11.0% ($P<0.01$) and systolic cell shortening was 209.6 + 13.4% ($P<0.001$) of basal values, respectively ($n=10$ each). These values were presented as the control responses to ET-1 in Figure 3 (left-hand side columns).

ISO at 10 nM induced a pronounced increase in cell shortening associated with a remarkable elevation of the amplitude of Ca^{2+} transients (Figure 2a). In addition, the increase in cell shortening induced by ISO was associated with a marked abbreviation of the duration of cell shortening and that of Ca^{2+} transients (Figure 2b). After washout for 15 min, the peak cell shortening and peak Ca^{2+} transient returned to the basal levels (Figure 2a). On average, diastolic indo-1 ratio and diastolic cell length were not significantly affected by ISO at 10 nM (Table 1) while the systolic indo-1 ratio and systolic cell shortening in these myocytes were increased significantly: when the basal values were assigned to 100% systolic indo-1 ratio was 204.0 + 9.1% ($P<0.001$) and systolic cell shortening was 191.1 + 14.8% ($P<0.001$) of basal values, respectively ($n=8$, each). These values were presented as the control responses to ISO in Figure 4 (left-hand side columns). The increase in systolic cell shortening induced by ISO at 10 nM was equivalent to that induced by ET-1 at 0.1 nM, but the increase in the amplitude of indo-1 ratio induced by ISO was significantly greater ($P<0.01$) than that induced by ET-1.

Influence of HOE 642 and KB-R9032

In this series of experiments, we first studied the direct effects of HOE642 and KB-R9032 on the baseline levels of indo-1 ratio and cell shortening by means of cumulative administration of HOE642 and KB-R9032. HOE642 at concentrations of $10^{-7} \sim 10^{-4}$ M did not affect the baseline indo-1 ratio and cell shortening (1.8 mM $[Ca^{2+}]_o$). KB-R9032 at $10^{-7} \sim 10^{-6}$ M did not significantly affect the baseline indo-1 ratio and cell shortening, but at 10^{-5} M, it inhibited significantly baseline cell shortening (76.1 ± 10.3% of the basal value; $P<0.05$, $n=6$), an indication that KB-R9032 at 10^{-5} M and higher concentration may elicit a nonselective inhibitory action.

HOE642 at 1 μM did not affect the amplitude of indo-1 ratio and systolic cell shortening. On average, the indo-1 ratio

Table 1 Changes in diastolic levels of cell length and indo-1 ratio induced by ET-1 (0.1 nM) and ISO (10 nM) in rabbit cardiomyocytes

	n	*Diastolic cell length* (μm)	*Diastolic indo-1 ratio*
Control	10	136.9 ± 5.5 (111.5–173.8)	1.02 ± 0.08 (0.75–1.54)
ET-1 0.1 nM	10	134.3 ± 5.3	1.06 ± 0.09
Control	8	130.6 ± 6.8 (111.0–172.1)	0.98 ± 0.09 (0.72–1.48)
ISO 10 nM	8	129.9 ± 6.7	1.04 ± 0.09

Values presented are means ± s.e.mean of diastolic cell length (μm) and indo-1 ratio. Numbers in parentheses represent range within which measured values were distributed.

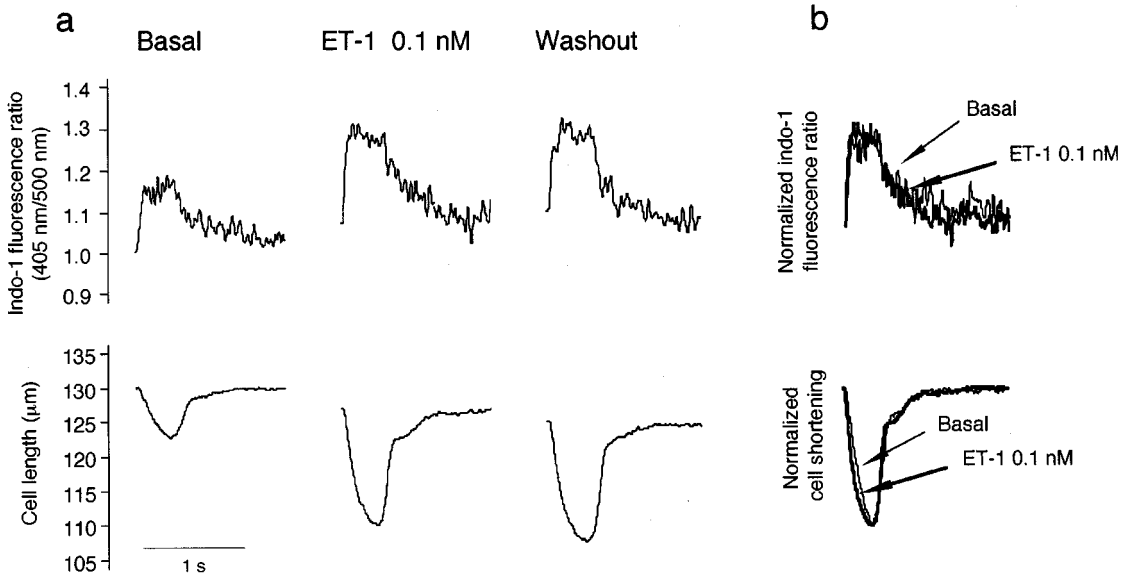

Figure 1 Effects of ET-1 at 0.1 nM on indo-1 fluorescence ratio (upper panel) and cell length (lower panel) in a rabbit single ventricular cardiomyocyte loaded with indo-1/AM. (a) actual tracings; (b) normalized superimposed tracings. Basal: baseline Ca^{2+} transient and cell shortening prior to administration of ET-1; washout: 15 min after washout.

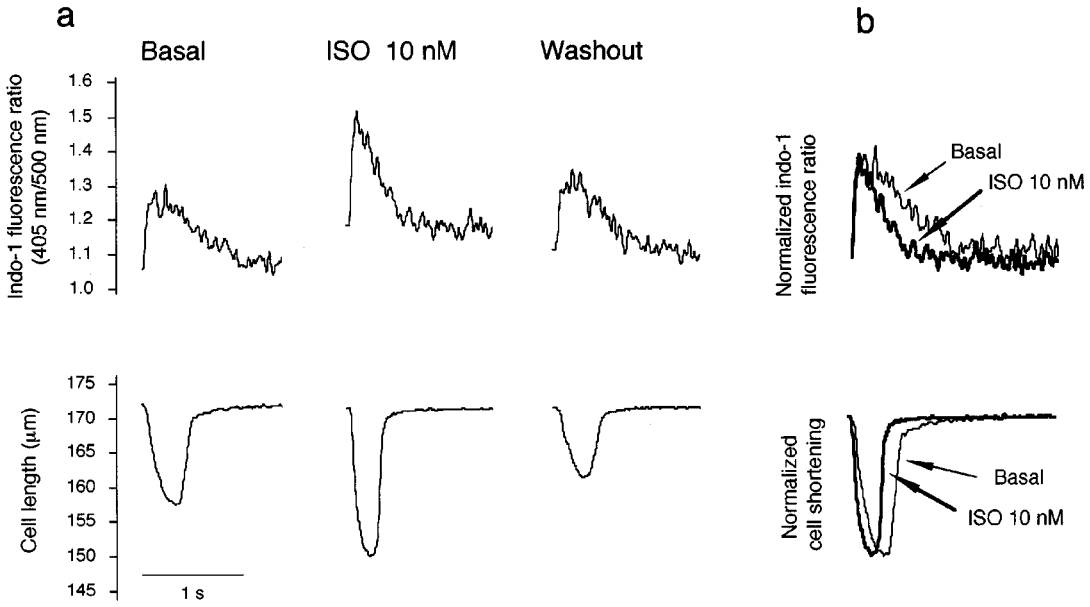

Figure 2 Effects of ISO at 10 nM on indo-1 fluorescence ratio (upper panel) and cell length (lower panel) in a rabbit single ventricular cardiomyocyte loaded with indo-1/AM. (a) actual tracings; (b) normalized superimposed tracings. Basal: baseline Ca^{2+} transient and cell shortening prior to administration of ISO; washout: 15 min after washout.

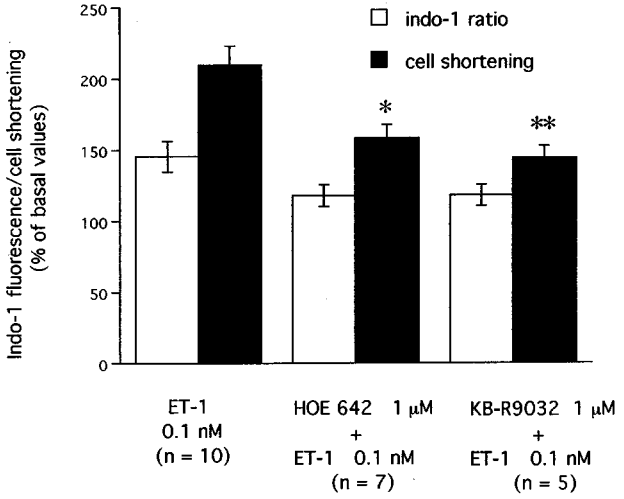

Figure 3 Influence of HOE642 (1 μM) and KB-R9032 (1 μM) on the effects of ET-1 at 0.1 nM on indo-1 fluorescence ratio and cell shortening in rabbit single ventricular cardiomyocytes loaded with indo-1/AM. HOE642 or KB-R9032 was applied 15 min prior to and during administration of ET-1. $*P<0.05$; $**P<0.01$ vs the control response to ET-1 in the absence of the inhibitors.

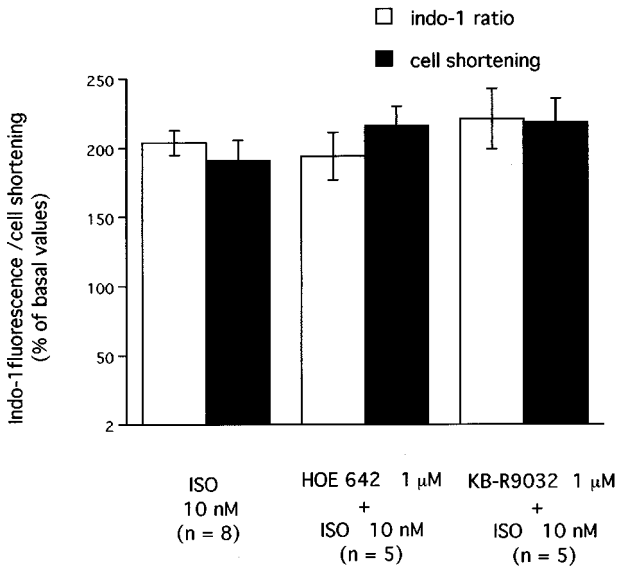

Figure 4 Influence of HOE642 (1 μM) and KB-R9032 (1 μM) on the effects of ISO at 10 nM on indo-1 fluorescence ratio and cell shortening in rabbit single ventricular cardiomyocytes loaded with indo-1/AM. HOE642 or KB-R9032 was applied 15 min prior to and during administration of ET-1.

and cell shortening were 106.3 ± 4.2 and $102.8 \pm 4.6\%$ of the basal values, respectively ($n=7$). ET-1 at 0.1 nM increased the indo-1 ratio and cell shortening in the presence of HOE642; however, the increase in systolic cell shortening induced by ET-1 was significantly less than the increase in the absence of HOE642 (Figure 3).

KB-R9032 at 1 μM did not affect the amplitude of indo-1 ratio and systolic cell shortening by itself: on average, the indo-1 ratio and cell shortening were 97.6 ± 9.0 and $96.6 \pm 6.4\%$ of the basal values, respectively ($n=5$). The increase in systolic cell shortening induced by ET-1 was significantly decreased by KB-R9032, but the increase in the amplitude of indo-1 ratio induced by ET-1 was not significantly different from that in the presence of KB-R9032 (Figure 3).

The increases in the amplitude of indo-1 ratio and cell shortening induced by ISO at 10 nM were unaffected either by HOE642 or by KB-R9032 (Figure 4).

Discussion

ET-1 at 0.1 nM and ISO at 10 nM elicited an increase in cell shortening in association with an increase in the amplitude of Ca^{2+} transients in isolated single rabbit ventricular cardiomyocytes. While the increases in systolic cell shortening induced by ET-1 and ISO were equivalent, the increase in the amplitude of indo-1 ratio induced by ET-1 was smaller than that induced by ISO. These results are essentially consistent

with the previous findings that ET-1 induced an increase in myofibrillar Ca^{2+} sensitivity in the ferret (Wang *et al*., 1991), rat (Kelly *et al*., 1990) and rabbit (Fujita & Endoh, 1996; Yang *et al*., 1999) ventricular myocardium and cardiomyocytes.

ET-1 did not affect appreciably the time course of indo-1 ratio and cell shortening (Figure 1), but ISO abbreviated these parameters prominently (Figure 2). The abbreviation of Ca^{2+} transients leads to the shift of [Ca^{2+}]-shortening (or force) relationship to the right (Yue, 1987; Endoh & Blinks, 1988). It has to be noted, therefore, that the difference in the relationship between ET-1 and ISO includes the shift of the relationship to the direction of apparent decrease in Ca^{2+} sensitivity (rightward shift) due to abbreviation of Ca^{2+} transients induced by ISO, in addition to the increase in Ca^{2+} sensitivity induced by ET-1.

Angiotensin II may elicit a PIE through an identical signalling process to ET-1 in rabbit ventricular cardiomyocytes (vide infra). Angiotensin II at 0.1 μM that elicited the maximal response increased the cell shortening to $206.2 \pm 7.2\%$ and the indo-1 ratio to $138.8 \pm 6.5\%$ ($n=6$), when the baseline values were assigned to 100% (Fujita & Endoh, 1999). These responses are equivalent to those induced by ET-1 at 0.1 nM ($209.6 \pm 13.4\%$; $145.6 \pm 11.0\%$, respectively) in the current study. Corresponding percentage increases induced by ET-1 at 0.1 nM in rabbit ventricular myocytes in the previous study (Yang *et al*., 1999) were slightly greater than those in the present study: 246% in cell shortening and 171% in indo-1 ratio, respectively, which may be within the range of individual variations. In isolated rabbit papillary muscles, the maximal PIEs of ET-1, angiotensin II and the α_1-adrenoceptor agonist, all of which act in association with acceleration of PI hydrolysis, were $60 \sim 70\%$, $40 \sim 50\%$ and $50 \sim 60\%$ of the maximal PIE of ISO, which show a slight variation but are considered to be essentially equivalent to each other (Endoh *et al*., 1996a).

The increase in cell shortening and indo-1 ratio induced by ET-1 was not reversible with washout for 15 min which was in strong contrast to the observation that the response to ISO or elevation of $[Ca^{2+}]_o$ was readily reversed with washout. The reason for irreversibility of the ET-1-induced response may be ascribed to the characteristics of interaction of ET-1 with its binding sites: the specific [^{125}I]-ET-1 binding to the membrane fraction of rabbit ventricular myocardium was scarcely reversed with washout or scarcely displaced by cold ligands once the binding occurs (Endoh *et al*., 1996b). The PIE of ET-1 lasted even after washout for 2 h (Kasai *et al*., 1994). By contrast the PIE of angiotensin II was readily reversed with washout in rabbit papillary muscles (Ishihata & Endoh, 1995; Talukder & Endoh, 1997). While these observations imply that the specific binding and PIE of ET-1 may be more prolonged than other agonists, including Angiotensin II and ISO in rabbit cardiomyocytes and papillary muscle, further research will be required to elucidate whether these pharmacological characteristics obtained in *in vitro* (*ex vivo*) experiments are reflected to the ET-1-induced cardiac effects *in vivo*.

Novel selective inhibitors of Na^+/H^+ exchanger, HOE624 (Scholz *et al*., 1995) and KB-R9032 (Yamamoto *et al*., 1998), decreased significantly the increase in cell shortening induced by ET-1 in rabbit ventricular cardiomyocytes (Figure 4). Since these compounds did not affect the baseline level of cell shortening and the increase in cell shortening induced by ISO, the inhibition of the ET-1-induced effect is considered to be due to their selective inhibitory action on the activation of Na^+/H^+ exchanger. These results are on line with the findings that the classical inhibitor of Na^+/H^+ exchanger 5-(N-ethyl-N-isopropyl) amiloride (EIPA) at 0.3 and 1 μM inhibited the angiotensin II-induced responses of indo-1 ratio and systolic cell shortening in rabbit ventricular cardiomyocytes (Fujita & Endoh, 1999). In these experiments, however, it became evident that EIPA at 1 μM and higher interfered with detection of indo-1 ratio by an increase in autofluorescence of the myocyte. In this respect novel inhibitors have advantage that they did not elicit such an effect as EIPA on autofluorescence.

ET-1 as well as angiotensin II and phenylephrine have been shown to stimulate the PI hydrolysis in cardiac muscle (Otani *et al*., 1988; Galron *et al*., 1990; Fedida *et al*., 1993; Endoh, 1994), and thereby these agonists share a common signal transduction pathway that involves the activation of PKC by DAG (Nishizuka, 1988) and release of Ca^{2+} ions from intracellular stores by IP_3 (Berridge, 1993). In cardiac myocytes, the experimental evidence is lacking that supports the functional significance of Ca^{2+} release induced by IP_3 in contractile regulation, because Ca^{2+}-induced Ca^{2+} release mechanism plays a central role in cardiac E-C coupling (Fabiato & Fabiato, 1978; Endoh, 1994). On the other hand, the activation of PKC might contribute to regulation of cardiac Ca^{2+} signalling to lead to the PIE of the receptor agonists that stimulate PI hydrolysis. It has been suggested that ET-1, angiotensin II and α-adrenoceptor agonists may stimulate the sarcolemmal Na^+/H^+ exchange *via* PKC-mediated pathway, leading to intracellular alkalinization and increase in myofibrillar Ca^{2+} sensitivity (Krämer *et al*., 1991; Matsui *et al*., 1995). In addition, the potential increase in intracellular Na^+ ions that results from activation of Na^+/H^+ exchanger may indirectly lead to an increase in $[Ca^{2+}]_i$ *via* Na^+/Ca^{2+} exchanger in cardiac tissue (Iwakura *et al*., 1990). Supporting the latter postulate, we have recently shown that an inhibitor of reverse-mode Na^+/Ca^{2+} exchange KB-R7943 elicited a selective inhibitory action on the PIE of ET-1 in adult rabbit ventricular myocytes (Yang *et al*., 1999). In these experiments, however, we have noticed that the ET-1-induced increase in cell shortening remains less inhibited even when the ET-1 induced increase in Ca^{2+} transients has been abolished by KB-R7943 (Yang *et al*., 1999). These observations indicate that mechanisms other than activation of Na^+/Ca^{2+} exchange are involved in the inotropic regulation induced by ET-1. The present findings with novel inhibitors of Na^+/H^+ exchanger provide a strong support for the role of the ion exchanger in an increase in myofibrillar Ca^{2+} sensitivity to contribute in part to the PIE of ET-1.

The increase in Ca^{2+} transients induced by ET-1 was slightly reduced by HOE642 or KB-R9032, but the difference was not significant compared with the control, an indication that mechanisms other than the ion exchanger are involved in the ET-induced increase in Ca^{2+} transients. While ET-1 increases L-type Ca^{2+} current in voltage-clamped rabbit ventricular myocytes (Watanabe & Endoh, 1999), the facilitatory effect of ET-1 on L-type Ca^{2+} current was trivial in general compared with the effect of β-adrenoceptor stimulation in mammalian cardiac muscle (Vigne *et al*., 1990; Cheng *et al*., 1995). Therefore it is supposed that the indirect increase in Ca^{2+} influx due to prolongation of action potential duration induced by inhibition of K^+ channels might play a more important role than the direct effect on L-type Ca^{2+} in the increase in Ca^{2+} transients induced by the receptor agonists that stimulate the PI hydrolysis in cardiac myocytes (Fedida *et al*., 1993; Watanabe & Endoh, 1999).

In conclusion, the PIE of ET-1 may be due to contribution of both an increase in Ca^{2+} transients and an increase in myofibrillar Ca^{2+} sensitivity. Activation of Na^+/H^+ exchanger may play a crucial role in the PIE and the sensitization of myofilaments to Ca^{2+} ions induced by ET-1 in adult rabbit ventricular cardiomyocytes.

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